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An earlier report described the differences in the steam-volatile neutral substances in a series of flue-cured leaf samples consisting of two groups which were designated "Aromatic" or "Aroma-deficient" (Burdick et al., 1963). It was concluded that no correlation was observed between leaf aroma and the levels of total volatile neutrals² in the samples. However, recent acquisition of panel test findings on the relative aromaticity of these samples has permitted revision of the original conclusions since a tendency toward a relationship became apparent (Stedman et al., 1964); details on this point are discussed below. Although a similar tendency was observed between leaf aroma and total volatile leaf acids in these samples (Schmeltz et al., 1963; Stedman et al., 1964) some discrepancies were noted in studies linking smoke flavor and total volatile smoke acids in the smoke of cigarettes prepared from these samples (Stedman et al., 1964). The present report is an extension of this work and describes the total volatile neutral substances in the smoke of these cigarettes and their possible relationship with smoke flavor.

Experimental

The cigarettes, smoking conditions, and the initial fractionation of

the condensates have been previously described (Stedman et al., 1964, 1963). Bases and neutrals from the smoke of 45 cigarettes were ultimately obtained in an ether solution (125 ml) which was extracted with aqueous 1N HCl (5 times, 100 ml total) to remove bases. The resulting ether solution of neutrals was washed once with aqueous 0.5% NaOH and then with successive 5 ml portions of saturated NaCl solution until the washings were neutral. The ether solution of neutrals was steam distilled and both ether and aqueous distillate (1000 ml) were collected in a cooled receiver. NaCl was added to the ether-aqueous distillate mixture until the aqueous layer was saturated and, after vigorous shaking, the layers were separated into an ether solution (A) and an aqueous layer. The aqueous layer was extracted 5 times (750 ml total) with ether and all ether extracts were pooled with A. The combined ether extract was dried over MgSO₄ and concentrated to 0.5 ml (B) for gas chromatographic analysis. For each sample, an aliquot of concentrate (B) equivalent to 7-12 mg of residue was injected. The methods for solvent removal, residue determination, and gas chromatographic separations on a Carbowax 20M³ column have been described earlier (Burdick et al., 1963). However, in the present study, programming was stopped at 210° instead of 240°C to increase the life of the column.

For quantitative measurements, comparisons of "Equivalent Peak Area" (EPA) (Burdick and Stedman, 1963) were made. In the present study, EPA was calculated by

$$6.1 \times 10^4 A$$

EPA = $\frac{V}{VP}$ in which A was

VP

the measured peak area (cm^2), V was the volume of the injected aliquot (μl), and P was the average length (%) of the cigarette smoked. Such EPA values represent a theoretical peak area which would be obtained on injection of the entire quantity of steam-volatile neutral substances in the condensate resulting from smoking 55% of each of 100 cigarettes. Limitations on this analytical method and on the present series of studies in general have been discussed previously (Stedman et al., 1964).

Results and Discussion

Except as noted below, the chromatograms of the volatile neutral fraction of all smoke condensates were qualitatively similar to those previously obtained from smoke condensates of blended and unblended cigarettes. For convenience, the same system of numbering peaks used in the previously shown chromatogram (Burdick and Stedman, 1963) was employed. Peaks 4, 5, 8, and 9 contained in part extraneous substances derived from the solvent (ether). The change in programming conditions from the previous study resulted in reduced resolution of those substances eluting after peak 52 but separations were satisfactory for quantitative comparisons. The previously found peaks 61-

¹Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

²The terms "Total Volatile Neutrals" and "Total Volatile Acids" refer to the substances determined by the gas chromatographic methods employed in this work.

³Mention of product or company names does not constitute endorsement by the Department of Agriculture over others not named.

Table 1. Quantitative differences in certain steam-volatile neutral components of smoke condensates of cigarettes made from aromatic or aroma-deficient tobacco.

Peak No.	EPA/100 cigarettes*	Aromatic cigarettes			Relative amount**	
		A	B	C	A	B
4	20	1.0	2.9	2.5	1.4	1.3
5	9	1.0	3.0	4.3	2.0	3.4
6	45	1.0	1.0	.66	.85	.34
7	25	1.0	1.6	1.5	.96	1.6
8	289	1.0	1.5	.87	1.1	.83
9	265	1.0	7.5	.42	.62	.37
10	61	1.0	1.8	3.1	1.2	2.0
11	16	1.0	1.8	1.6	1.3	3.5
12	130	1.0	2.1	2.6	1.1	3.3
13	67	1.0	2.4	2.5	2.2	2.7
14	22	1.0	6.5	2.9	2.1	12
15	7	1.0	5.0	4.1	7.0	5.5
16	49	1.0	2.3	1.8	1.9	4.1
17	386	1.0	1.4	1.6	1.2	2.2
17a	58	1.0	1.3	1.0	1.1	1.2
18a	38	1.0	1.6	1.4	1.1	1.5
18b	204	1.0	1.4	1.7	1.0	1.4
18	150	1.0	1.4	1.8	.94	1.4
19	72	1.0	2.0	1.8	1.4	1.8
20	92	1.0	2.2	1.8	1.4	1.7
21	305	1.0	1.6	1.8	1.0	2.2
22	16	1.0	1.4	.86	1.1	.70
23	142	1.0	1.2	1.5	1.0	3.6
24	788	1.0	1.5	1.7	.95	1.6
25	119	1.0	1.8	1.8	1.1	1.5
25a	76	1.0	1.6	1.6	1.3	1.5
26	79	1.0	1.8	1.7	1.2	2.3
27	36	1.0	1.9	.47	.78	2.1
28	61	1.0	1.3	1.2	.53	1.3
29	18	1.0	.37	.25	.43	.61
30	38	1.0	1.1	.36	.49	.52
31	14	1.0	1.2	3.0	.92	4.1
32	85	1.0	1.2	1.4	.51	1.3
33	29	1.0	.61	.62	.53	.53
33a	20	1.0	1.7	1.1	.62	1.6
34	14	1.0	.41	.51	.32	.82
35	166	1.0	1.6	1.6	.39	1.8
36	9	1.0	.50	.76	.74	1.7
37	128	1.0	1.3	1.3	.54	.84
37a	72	1.0	.81	1.1	.51	.89
38	54	1.0	.77	.89	.49	.61
40	267	1.0	1.2	1.3	.39	.98
41	101	1.0	.64	.50	.46	.57
42	336	1.0	1.0	1.2	.50	.91
43	103	1.0	.87	.99	.45	.85
44	211	1.0	.75	.80	.42	.46
45	72	1.0	.81	.98	.52	.49
46	285	1.0	.68	.77	.39	.36
47	386	1.0	.67	.85	.57	.48
48	47	1.0	.57	.63	.54	.47
49	85	1.0	.63	.75	.59	.46
50	54	1.0	.50	.89	.39	.29
51	56	1.0	2.2	.85	.37	.55
52	1211	1.0	.77	.89	.53	.48
53	20	1.0	1.1	1.9	.61	1.9
54	283	1.0	.63	.72	.34	.48
55	361	1.0	.45	.53	.41	.25
56	74	1.0	.57	.98	.45	.18
57	67	1.0	.74	1.3	.55	.36
58	79	1.0	.20	.29	.08	<.01
59	99	1.0	.27	.09	.26	<.01
60	65	1.0	.02	.04	.24	<.01
EPA (all peaks)	8531	1.0	1.3	1.2	.72	2.3
EPA (peaks 35-60)	4691	1.0	.78	.88	.46	.56

*For Aromatic A cigarettes. See text for explanation of Equivalent Peak Area (EPA).

**Values for EPA per 100 cigarettes of indicated tobacco.

**Values for EPA per 100 cigarettes of Aromatic A tobacco.

Table 2. Comparisons of leaf aroma and total selected volatile neutrals in leaf and smoke of bright tobaccos.

Sample	Relative leaf aroma*	Steam-volatile neutrals**		
		Leaf	TVN	Smoke Peaks 35-60
Aromatic A	1	1.0	1.0	1.0
Aromatic B	3	.57	1.3	.78
Aromatic C	2	.62	1.2	.88
Aroma-deficient A	4	.57	.72	.46
Aroma-deficient B	5	.24	2.3	.56

*Determined by panel of 15 observers. 1 = highest aromaticity.

**In relative amounts. Leaf data are based on total volatile neutrals (TVN) from previous publication (Burdick *et al.*, 1963). Smoke data are from Table 1.

vor and the high-boiling neutrals discussed above.

In determining the volume of concentrate to inject for chromatographic analysis a residue weight was obtained by evaporating 50 μ l the concentrate under a gentle stream of N_2 for exactly 5 minutes as previously described. In the case of leaf the published values (Burdick *et al.*, 1963) for such residue weights show some relationship with the relative aromaticity of the leaf. In the case of smoke, the values for the residue weights for Aromatic A, Aromatic B, Aromatic C, Aroma-deficient A, and Aroma-deficient B were 274, 283, 295, 173 and 217 mg/100 cigarettes, respectively. Although not an exact correlation, the aromatic samples were all larger than the aroma-deficient (Table 3). Chromatography of an amount of residue equal to that present in the ethereal neutral concentrate showed that peaks 40-60 were not appreciably diminished in size when the solvent was evaporated during residue determination. However, those peaks eluting before peak 35 were either absent or considerably diminished in size. Some relationship among the amounts of these higher boiling neutral substances, the residue weights of the neutral concentrates, and the relative smoke flavor might therefore be expected.

The levels of total volatile acids (C_2 to approximately $n-C_7$) in the smoke condensates of these cigarettes have been previously reported (Stedman *et al.*, 1964). Ratios of total neutrals to total volatile acids did not show any relationship with smoke flavor (Table 4). However, when the ratios of the selected high-boiling neutrals (peaks 35-60) to total volatile acids were compared, the smoke from all aromatic cigarettes gave values larger than the aroma-deficient samples.

It is of interest that a definite fruit-like or sweet aroma was observed when these higher boiling neutral substances emerged from the exit port of the gas chromatograph. The aroma was particularly noticeable when peak 52 emerged. This peak has been shown to contain neophytadiene, a non-aromatic substance (Burdick and Stedman, 1963). However, as previously noted, the unsymmetrical shape of this peak indicated that additional components are present which may be aromatic. It should be noted that this peak comprises more than 25% of the EPA attributed to the higher boiling substances (Table 1) and that the

Table 3. Comparison of smoke flavor and total or selected steam-volatile neutral substances in smoke of cigarettes made from aromatic or aroma-deficient tobaccos.

Sample	Smoke* flavor	Relative Ratings	
		Total** neutrals	Selected** neutrals
Aromatic A	1-2	4	1
Aromatic B	3	2	3
Aromatic C	1-2	3	2
Aroma-deficient A	4	5	5
Aroma-deficient B	5	1	4

*Relative flavor determined by panel testing. 1 = highest flavor, 1-2 = two samples were of equal flavor.

**Based on total EPA of all peaks ("Total neutrals") or of peaks 35-60 ("Selected neutrals") from Table 1. 1 = largest amount in the five samples.

56 were either absent in the sample or did not elute under the altered conditions of programming. In some cases, resolution was improved over the previous chromatograms so that distinct inflections on peaks could be discerned, e.g. peaks 17, 18a, b, 25a, 33a, and 37a. Peak 39 did not resolve and was not considered herein. Peaks 1-3 were very small in the present series of samples and were not quantitatively evaluated. Tentative identifications for some peaks have been given previously (Burdick and Stedman, 1963).

Table 1 shows the total EPA values, EPA values per peak for Aromatic A, and ratios of EPA values for all peaks and for certain selected peaks using Aromatic A as the reference. The decreasing order of total EPA (all peaks) was Aroma-deficient B, Aromatic B, Aromatic C, Aromatic A, and Aroma-deficient A. The large EPA for Aroma-deficient B was due mainly to large amounts of peaks eluting early, i.e. peaks 4-36; however, the smoke of both aroma-deficient tobaccos showed smaller amounts of most of the higher-boiling substances, i.e. the remainder of the peaks. These latter substances elute in the same range

that the major components of the steam-volatile neutrals of leaf elute and, in fact, include some of the leaf components (Burdick *et al.*, 1963).

Table 2 shows a comparison of the total or selected steam-volatile neutrals in leaf and smoke and, in addition, includes data on the relative ratings of leaf aroma. As noted above, the latter data were not available at the time of our original publication. There appeared to be some tendency toward a relationship between the relative leaf aromaticity and the total leaf neutrals, although Aromatic B and Aroma-deficient B were similar. Comparing leaf aroma and total smoke neutrals, there appeared to be little relationship; however, by limiting the neutrals to peaks 35-60, some tendency toward a correlation developed although the aroma-deficient samples were reversed.

Attempts to correlate smoke flavor, as determined by a panel of 22-25 smokers, with the analytical data obtained in this study are summarized in Table 3. No simple correlation was observed between smoke flavor and total volatile neutrals in all peaks of Table 1. However, some trend was noted between smoke fla-

Table 4. Comparison of smoke flavor and ratios of neutral substances to total acids for aromatic or aroma-deficient cigarette smoke condensates.

Cigarette	Smoke* flavor	Relative amounts**			
		Total acids (A)	Total neutrals (B)	Selected neutrals (C)	B/A
Aromatic A	1 - 2	1.0	1.0	1.0	1.0
Aromatic B	3	.69	1.3	.78	1.9
Aromatic C	1 - 2	.82	1.2	.88	1.5
Aroma-deficient A	4	.66	.72	.46	1.1
Aroma-deficient B	5	.90	2.3	.56	2.6

*Relative flavor, 1 = highest flavor.

**Based on mg/100 cigarettes (Total acids) or EPA (Neutrals). See table 3 for explanation of neutrals.

relative amounts of this peak in the five samples correlate reasonably well with the relative smoke flavor.

Summary

Using a previously described gas chromatographic method, the relative amounts of neutral substances were studied in the smoke of bright cigarettes made from tobaccos of different aromaticity. The analytical results were compared with panel test findings on the relative flavor of the cigarettes. Some tendency toward a relationship was found between certain higher boiling neutral components and smoke flavor; among these components was a peak containing neophytadiene and other substances which may be of special importance in this regard. No correlation was apparent when total neu-

tral components were evaluated. Relationships between the ratios of neutral substances to total acids and relative smoke flavor were also discussed.

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